

AMENDMENTS TO THE CLAIMS

1. **(Currently amended)** An isolated recombinant human arginase I, comprising having substantially the same amino acid sequence as set forth in Fig. 2C SEQ ID NO: 9 and having a purity of 80-100%.
2. **(Currently amended)** The recombinant human arginase I according to claim 1 further having comprising six additional histidines attached to the amino terminal end thereof.
3. **(Currently amended)** The recombinant human arginase I according to claim 1 or 2 having a specific activity of at least 250 I.U./mg.
4. **(Original)** The recombinant human arginase I according to claim 3 having a specific activity of 500 to 600 I.U./mg.
5. **(Currently amended)** The recombinant human arginase I according to claim 4, comprising a modification resulting that results in an *in vitro* plasma half-life of at least approximately 3 days.
6. **(Currently amended)** An isolated recombinant human arginase I according to claim 1, or 2 having a purity of at least 90%.
7. **(Original)** The recombinant human arginase I according to claim 5, wherein said modification is pegylation.
8. **(Original)** The recombinant human arginase I according to claim 7, wherein said pegylation results from covalently attaching at least one polyethylene glycol (PEG) moiety to said arginase using a coupling agent.
9. **(Original)** The recombinant human arginase I according to claim 8, wherein said coupling agent is selected from the group consisting of 2,4,6-trichloro-s-triazine (cyanuric chloride, CC) and succinimide propionic acid (SPA).
10. **(Currently amended)** A method of producing recombinant protein comprising:
 - (a) cloning a gene encoding said protein;
 - (b) constructing a recombinant *Bacillus subtilis subtilis* strain for expression of said protein;

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(c) fermenting said recombinant *Bacillus subtilis* cells using fed-batch fermentation;

(d) heat-shocking said recombinant *Bacillus subtilis* cells to stimulate expression of said recombinant protein; and

(e) purifying said recombinant protein from the product of said fermentation.

11. **(Original)** The method according to claim 10 wherein said *Bacillus subtilis* is a prophage.

12. **(Currently amended)** The method according to claim 10 or 11 wherein said protein is human arginase I.

13. **(Currently amended)** The method according to claim 12 wherein said human arginase I ~~has~~comprises six histidines linked to the amino-terminus thereof, and said purifying step comprises affinity chromatography in a chelating column.

14. **(Original)** The method according to claim 12 wherein said fermenting step is performed using a feeding medium consisting essentially of 180-320 g/L glucose, 2-4 g/L MgSO₄•7H₂O, 45-80 g/L tryptone, 7-12 g/L K₂HPO₄ and 3-6 g/L KH₂PO₄.

15. **(Original)** A pharmaceutical composition comprising an isolated and substantially purified arginase.

16. **(Original)** The pharmaceutical composition according to claim 15 wherein said recombinant human arginase is human arginase I.

17. **(Currently amended)** The pharmaceutical composition according to claim 15 wherein said recombinant human arginase is human arginase I, further comprising containing six additional histidines attached to the amino terminal end thereof.

18. **(Original)** The pharmaceutical composition according to claim 15, wherein said composition is further formulated in a pharmaceutically acceptable carrier.

19. **(Currently amended)** The pharmaceutical composition according to claim 15, wherein ~~said~~the formulation of said pharmaceutical composition is in a form suitable for oral use, for a sterile injectable solution or a sterile injectable suspension.

20. **(Original)** The pharmaceutical composition according to claim 16, wherein said recombinant human arginase I has a specific enzyme activity of at least 250 I.U./mg.

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21. (Original) The pharmaceutical composition according to claim 20, wherein said recombinant human arginase I has a specific enzyme activity of 500 to 600 I.U./mg.

21.22. (Currently amended) The pharmaceutical composition according to claim 16, wherein said recombinant human arginase I has a half-life in ~~said~~-patient plasma of at least 3 days.

22.23. (Currently amended) The pharmaceutical composition according to claim 21 ~~22~~, wherein said recombinant human arginase I has a half-life in ~~said~~-patient plasma of approximately at least 1 day.

23.24. (Currently amended) ~~The use of A method of treatment of human malignancies, comprising administering human arginase I of claim 1 for the preparation of a medicament.~~

24.25. (Currently amended) ~~The use according to claim 23 wherein said medicament is used for the A method of treatment of human malignancies, comprising administering the pharmaceutical composition of claim 15.~~

25.26. (Currently amended) ~~The use according to method of claim 24-25, wherein said human malignancies are selected from the group consisting of: liver tumour-tumor, breast cancer, colon or cancer and rectal cancer.~~

26.27. (Currently amended) A method of treatment of human malignancies comprising administering recombinant human arginase ~~into to~~ a patient.

27.28. (Currently Amended) A method of treatment of human malignancies in a patient comprising administering a pharmaceutical composition that reduces the physiological arginine level in said patient to below 10 μ M for at least 3 days.